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TraT Lipoprotein, a Plasmid-Specified Mediator of Interactions between Gram-Negative Bacteria and Their Environment

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INTRODUCTION

Interactions between gram-negative bacteria and their immediate environment are mediated by components present on the extracellular face of the outer membrane (48, 49, 63) (Fig. 1). These components play critical roles in many of the recognition events between the cell and its surroundings and are of particular importance in the disease process, in which pathogenic bacteria interact with external factors produced by eucaryotic cells. Examples of diverse recognition processes involving cell surface components include binding of bacteriophages or bacteriocins, conjugal transfer of DNA, adhesion of bacteria to surfaces (e.g., eucaryotic cells), and nonproductive binding of host defense proteins such as complement and antibodies.

The outer membrane also serves as a permeability barrier, effectively excluding hydrophobic compounds but allowing the influx and efflux of small hydrophilic molecules (64, 89). In enteric bacteria, whose normal habitat is the intestine of warm-blooded animals, the permeability barrier is particularly efficient since their environment regularly contains a wide range of potentially toxic compounds, e.g., bile salts, lysophosphatides, lysozyme, phospholipases, and proteases, that rapidly reduce the viability of other microorganisms (18, 69). In addition to excluding these noxious agents, outer membrane components exposed on its external face must also function in their presence. The cell surface-exposed proteins might therefore be expected to have rather unusual structures and properties to allow their continued survival and function in a harsh environment.

The TraT lipoprotein is an example par excellence of a cell-surface-exposed protein involved in interactions between a bacterial cell and its surroundings. The protein is determined by the *traT* genes of large, usually conjugative plasmids and prevents a cell harboring a related plasmid from acting as a conjugal recipient when mated with donor cells carrying similar plasmids (1, 100). This phenomenon, termed surface exclusion, prevents unproductive mating between cells bearing the same plasmid and hence is likely to restrict the horizontal transfer of genetic information by plasmids to cells bearing closely related plasmids.

With the observation that the TraT protein increases the survival of host cells in serum (58) and the recent finding that the *traT* gene is present in isolation from other *tra* genes in virulence-associated plasmids of *Salmonella* and *Yersinia* species (91; B. China, T. Michiels, and G. R. Cornelis, Mol. Microbiol., in press), studies on the structure and function of the protein have increased in significance. Although the mechanism of action of the protein and its precise role in virulence remain to be elucidated, the available evidence suggests that TraT increases the resistance of bacteria to the lytic action of complement (58, 68, 77). This type of resistance is one of the major factors in the virulence of invasive bacteria that cause generalized infections (38, 39, 94), and hence an understanding of the activity mediated by TraT at the molecular level may be of general relevance.

A final aspect of the TraT protein concerns its use as a research tool, particularly in investigations of the special permeability properties of the outer membrane (62, 64, 89). The protein has a major advantage in such studies since its presence is not essential to the cell under most circumstances; this greatly facilitates the production and analysis of permeability mutants. The protein differs from other major outer membrane proteins (such as murein lipoprotein, OmpA protein, and the porins) in that it contains clearly defined hydrophobic domains. Despite these differences, however, studies of TraT permeability mutants have provided useful information on the nature of the permeability barrier and should give general insights into the molecular organization of the outer membrane.

This review considers the role of the TraT protein in surface exclusion and serum resistance and discusses recent applications of the protein in probing the permeability barrier function of the outer membrane and transporting foreign antigenic determinants to the bacterial cell surface (I. M.

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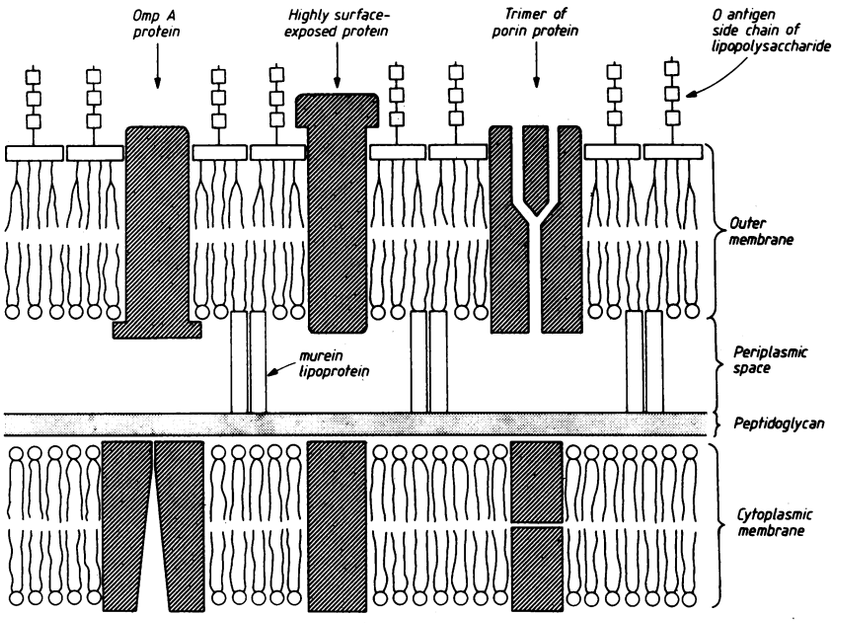

FIG. 1. Schematic representation of a section through the cell envelope of *E. coli* or *S. typhimurium*. In this diagram, enzymes present in the periplasmic space have been omitted for clarity and the O-antigen side chain (not present in *E. coli* K-12) has been shortened. Additionally, only two of the three pores thought to be present in each porin trimer are visible. Other components that may be present at the cell surface include pili, fimbriae, and capsules, depending on the strain studied. (Adapted from reference 64 with permission.)

Taylor, J. L. Harrison, and C. D. O'Connor, Mol. Microbiol., in press). Although the review focuses on one specific component of the outer membrane, it necessarily impinges on several other areas of research owing to the diverse activities and applications of the protein. Generally, these areas have been covered elsewhere in recent reviews (20, 35, 39, 63–65, 81, 89, 94, 103). For conciseness, therefore, only information of direct relevance is included here.

PHYSICOCHEMICAL PROPERTIES OF THE TraT PROTEIN

The F *traT* gene codes for a basic protein (pI ca. 9) of 23,709 daltons that is present at 20,000 to 30,000 copies per cell (1, 51, 56). However, the available evidence suggests that the protein in the outer membrane is an aggregate of TraT subunits (51, 57). Not only are results from genetic complementation experiments most readily explained by invoking an oligomeric structure, but also studies with purified preparations show that this form of the protein inhibits mating when added to mixtures of donor and recipient cells (57). Intriguingly, electron microscopy studies have shown that the purified, native TraT protein specified by F is a multisubunit, doughnut-shaped molecule, with an outer diameter of 18.5 nm and a central region appropriate for binding the F pilus (E. G. Minkley, Jr., unpublished results cited in reference 35). However, the precise number of subunits in the oligomeric form has not yet been determined.

In keeping with its outer membrane location, the TraT protein has several properties in common with other major outer membrane proteins, particularly the porins. Therefore, the oligomeric form is highly resistant to denaturation at temperatures below 70 to 80°C, to solubilization by a variety of detergents, and to digestion by trypsin or pronase in outer membrane fractions (51, 57). These properties suggest that the native protein is very tightly packed and hence resistant to penetration by denaturants or proteases. In view of the shared characteristics, it is rather surprising that there is virtually no homology between the amino acid sequences of these proteins (see "Comparison of the TraT Proteins" below). A comparison of their hydropathy plots and predicted secondary structures also shows major differences (Fig. 2). For example, the TraT protein is predicted to have substantially more α-helical content than either OmpF, OmpC, or LamB, which, like OmpA, have less than 15% α-helical structure (98), and TraT has two prominent stretches of uncharged residues (residues 99 to 110 and 118 to 135), in contrast to the much shorter hydrophobic segments in the other proteins. The TraT protein may therefore differ radically from these proteins in its molecular organization in the outer membrane.

Studies with lipopolysaccharide-deficient mutants of *Escherichia coli* have shown that in contrast to the OmpA or OmpF proteins, the TraT protein is stably maintained, even in strains totally lacking the neutral sugars in their lipopolysaccharide backbone (51). This observation has become more understandable with the discovery that the protein contains covalently bound fatty acids and hence is likely to be anchored into the membrane through lipid-lipid interactions as well as by protein-lipid interactions (71). The precursor form, which contains a signal sequence of 20 amino acid residues, is modified by the attachment of a thioether-linked diglyceride to the single cysteine residue in the protein (71). Two fatty acids are attached to the diglyceride via ester linkages and, following cleavage of the signal peptide by signal peptidase II, a further acyl group is attached to the cysteine residue (which forms the first amino acid of the mature protein) via an amide linkage. Gene fusion studies with the murein lipoprotein strongly suggest that only the first 10 or so amino acid residues of the mature portion of the protein are required for its targeting to the outer membrane (105), and it is highly likely that the same is

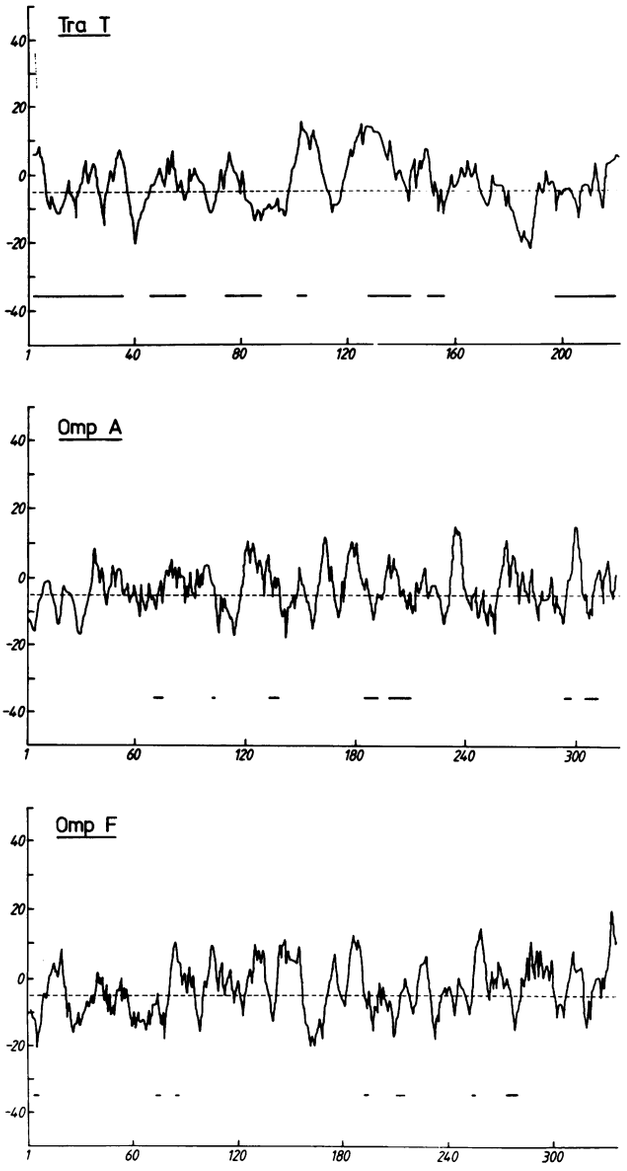

FIG. 2. Hydropathy profiles of the TraT, OmpA, and OmpF outer membrane proteins (mature forms). Regions predicted as likely to have α-helical conformation are also indicated (——) beneath each profile. The hydropathy index, averaged over seven residues, was plotted by the method of Kyte and Doolittle (47), and the α-helical content was calculated by the method of Garnier et al. (27). Overall, the TraT, OmpA, and OmpF proteins are predicted to have 55, 22, and 14% α-helical content, respectively.

true for other outer membrane lipoproteins such as TraT. The role of the two central hydrophobic regions in the TraT protein is unknown, but they presumably span the outer membrane and hence anchor the protein into it.

Membrane fractionation studies suggest that the precursor form of the protein can also be successfully translocated to the outer membrane (55). Therefore, the amide-linked fatty acid, which is added after proteolytic cleavage of the precursor, does not seem to be essential for maintenance of the protein in the membrane. It is probable, however, that the ester-linked fatty acids are important for anchorage, although this remains to be proved. Interestingly, the pro-TraT protein is also resistant to degradation by proteases; however, gel filtration studies suggest that processing is essential for conversion to the oligomeric and biologically active form of the protein (55).

Although a procedure for the purification of the F TraT protein has been published (57), it relied on a detergent that is no longer available. Therefore, further studies of the structure of the protein require an alternative method that allows purification of the protein to homogeneity. This has recently been developed by using a modification of a protocol for purification of the OmpF and OmpC porins (28; J. L. Harrison and C. D. O'Connor, unpublished results).

SURFACE EXPOSURE OF THE TraT PROTEIN AND ITS USE TO TRANSPORT FOREIGN ANTIGENS TO THE CELL SURFACE

The surface exclusion and serum resistance properties of the TraT protein indicate that at least part of the protein must be exposed at the cell surface (4, 54). This assumption was tested by Manning et al., who demonstrated that the tyrosine residues of the F and R6-5 proteins could be very efficiently labeled in whole *traT*$^+$ cells by using ^{125}I and lactoperoxidase (51, 54). Although the protein constituted only a minor band on a sodium dodecyl sulfate-polyacrylamide gel of whole-cell proteins, autoradiography showed that it was the most strongly labeled of all the bands. Now that the amino acid sequences of several major outer membrane proteins are available, it is clear that this result is not due to an unusually high concentration of tyrosine residues in the TraT protein. In fact, the F TraT protein has only seven tyrosines compared with 17, 22, and 29 tyrosines for the OmpA (16), LamB (34), and OmpF (12) proteins, respectively. Therefore, the available evidence suggests that a substantial portion of the TraT protein is exposed on the external face of the outer membrane.

Recently, the highly exposed nature of the TraT protein has been exploited to allow transport of foreign antigenic determinants (epitopes) to the bacterial cell surface (Taylor et al., in press). Hybrid TraT proteins in which the C3 epitope of the VP1 coat protein of type 1 poliovirus has been inserted into one of five sites in the protein (at amino acid residue 61, 125, 180, 200, or 216) were constructed. In three cases this led to exposure of the foreign antigen at the cell surface. Additionally, one derivative, with the C3 epitope inserted at residue 180 functioned in a genetic suppression assay (abolition of the SS-A phenotype; see the section on *traT* mutations that affect outer membrane permeability, below) and retained partial surface exclusion activity. This strongly suggests that the insertion of foreign antigenic determinants at this site does not grossly distort the structure of the protein. The ability of the TraT lipoprotein to transport and present foreign epitopes at the cell surface suggests that it might be useful in the construction of novel vaccine strains (for a review, see reference 20), particularly as it is known that lipopeptides efficiently prime virus-specific cytotoxic T lymphocytes in vivo (17). Although none of the hybrid proteins produced mediated detectable serum resistance, it will obviously be particularly important to assess the virulence properties of any vaccine strains produced in this way.

COMPARISON OF THE TraT PROTEINS

At present, the complete nucleotide sequences of the *traT* genes of F (37), R100 (68), Fo*lac* of *Salmonella typhi* (24), ColB-K98 (I. Taylor, K. Platt, and C. D. O'Connor, unpub-

lished EMBL accession number X14566), R6-5 (C. D. O'Connor and K. N. Timmis, unpublished results; EMBL accession number X52553), and the virulence plasmids of *Salmonella typhimurium* pSLT (91) and *Yersinia enterocolitica* pYV (China et al., in press) are known. Figure 3 shows a comparison of the amino acid sequences deduced from the DNA sequences. In each case the mature form of the TraT protein begins with a cysteine residue, to which the lipid moiety is added during the posttranslational modification process (71). For the F, pSLT, and pYV TraT proteins, lipid attachment has also been demonstrated by labeling studies (71; China et al., in press; our unpublished results).

The amino acid sequence of *Y. enterocolitica* TraT has 88% identity with that of F*olac* and 82% identity with the F and R100-1 proteins. The *S. typhimurium* TraT protein, on the other hand, is more similar to the protein encoded by R100-1 than to the *Y. enterocolitica* or F*olac* proteins. Therefore, there does not seem to be a specific class of TraT protein associated with the virulence plasmids.

Comparison of the primary structures of the proteins shows that both well-conserved and highly variable regions are present (Fig. 3). The former regions, in particular the NH_2- and COOH-terminal segments, are likely to correspond to structurally or functionally important parts of the protein. By contrast, the variable regions are less likely to be essential for the structural integrity of the protein but, by analogy with other outer membrane proteins, may correspond to surface-exposed portions of the protein (97). Three variable regions can be discerned, notably between amino acids 44 and 71, 88 and 102, and 157 and 161. Additionally, the region from 116 to 120 varies, although the amino acid alterations are more subtle. This portion of the protein has recently been shown to be important for the surface exclusion specificities of the proteins and is considered in more detail below (see the section on surface exclusion). The region, which is rich in Gly and Ala residues, also shows significant similarity to the C-terminal part of the adhesin proteins of phages T2 and K3 (79). Although the conserved and variable regions of the TraT proteins are now becoming clear, cloning and sequencing of more distantly related *traT* genes may allow the identification of other regions of structural or functional importance.

traT MUTATIONS THAT AFFECT OUTER MEMBRANE PERMEABILITY

Unlike other biological membranes, the outer membrane of gram-negative bacteria is generally impermeable to hydrophobic compounds (63, 64). An important consequence of this unusual property is that the bacteria are intrinsically resistant to many hydrophobic antibiotics that are highly effective against gram-positive organisms. Despite the large number of TraT molecules that generally accumulate in the

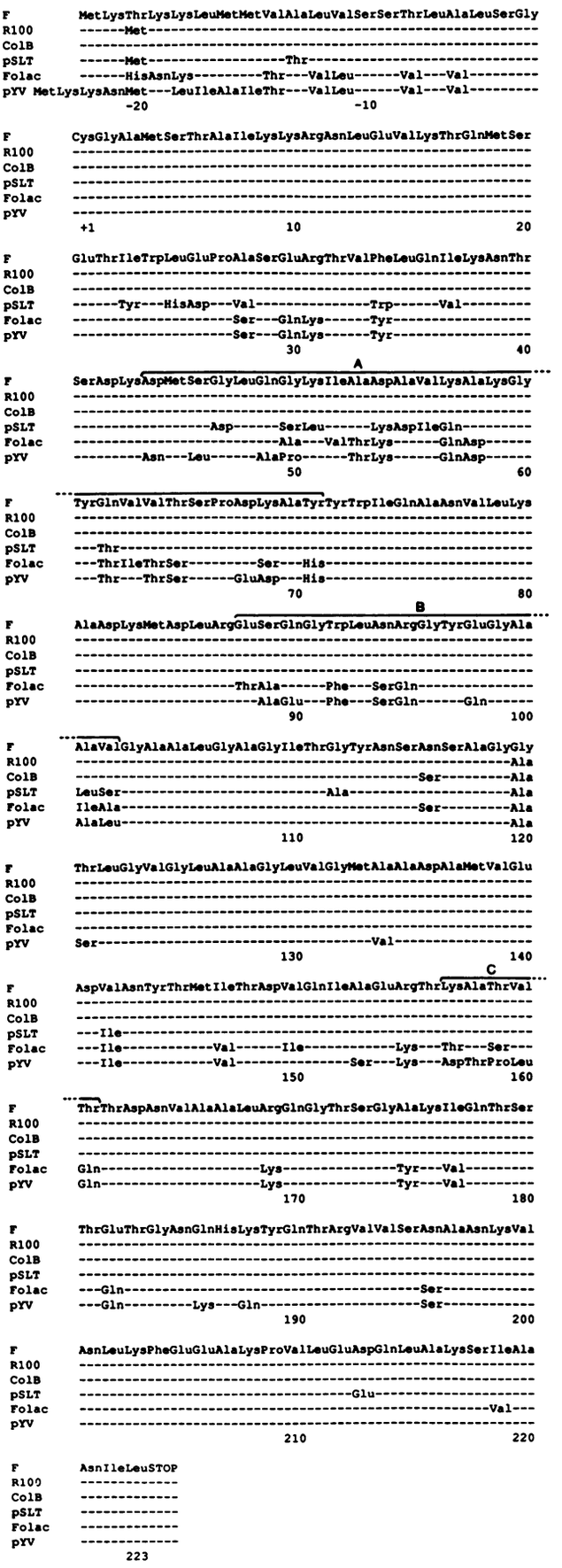

FIG. 3. Comparison of amino acid sequences of the F (37), R100 (68) F*olac* (24), ColB-K98 (Taylor et al., unpublished), pSLT (91), and pYV (China et al., in press) TraT proteins deduced from the gene sequences. The sequence of the *traT* gene carried by the plasmid R6-5 has also been determined (O'Connor and Timmis, unpublished), but since it is identical to that of the R100 gene, it is not included here. The Cys at position +1 is the first residue of the mature form of the protein and normally has lipid molecules covalently attached to it via thioether and amide linkages. In addition to the three variable regions marked A, B, and C, residues 116 to 120 are variable and determine the specificity of the TraT protein in surface exclusion (see text).

outer membrane when a cell gains a conjugative plasmid carrying the *traT* gene, the characteristic permeability properties of the membrane are unaffected (88). Overproduction of TraT from strong vector promoters, however, may be deleterious for cell growth (66).

The presence of a mutant TraT protein can increase the permeability of the outer membrane to hydrophobic compounds (76, 86). This was first shown by introducing short (linker) insertion mutations in the *traT* gene of R6-5 (86), cloned in a repressible vector system (66). The target site for the insertions was the amino acid residue Gly-125, which resides in a prominent hydrophobic region of the mature protein (Fig. 2). The insertions, which introduced negatively charged Asp or Glu residues into this region (owing to the insertion of an *Eco*RI or *Xba*I linker, respectively, into the *traT* gene), resulted in increased outer membrane permeability to hydrophobic antibiotics and detergents but not to hydrophilic compounds (86, 88). Additionally, the ability of the protein to increase serum resistance was diminished in these mutants (88).

An extensive study was performed to test the effect of insertion of 10 different amino acids by site-directed in vitro mutagenesis in the place of Leu-126 within the hydrophobic region of R6-5 TraT (91). There was no effect on membrane permeability when the inserted amino acids were nonpolar. However, if either positively (Arg or His) or negatively (Asp or Glu) charged amino acids were introduced, the bacteria became extremely sensitive to hydrophobic antibiotics. A similar phenotype was also caused by the insertion of Pro, probably owing to the disruptive effect of this residue in the region.

An independently isolated mutation increasing the permeability of the outer membrane of *S. typhimurium*, preliminarily called SS-A (90), was mapped to the virulence plasmid of *S. typhimurium* (87) and further shown to be located within the *traT* gene carried by the plasmid (76). Subsequently, determination of the nucleotide sequence of the mutated *traT* gene showed that the SS-A permeability phenotype was caused by a Gly-to-Arg alteration at position 132.

The antibiotic sensitivity of the SS-A mutant is corrected if a wild-type *traT* gene of F or R6-5 is introduced in *trans* (87). The presence of the TraT protein as an oligomer in the outer membrane (55) would explain the suppression of the SS-A phenotype in these merodiploid strains, assuming that the normal membrane integrity and barrier property are maintained if at least some subunits of the hetero-oligomer are wild type. The TraT protein produced by the plasmid Fo*lac* of *S. typhi* (21) did not restore the wild-type permeability barrier when introduced into the SS-A mutant (85), suggesting that the protein produced by Fo*lac* cannot form functional oligomers with the SS-A mutant proteins even though the amino acid sequences of the R100 and Fo*lac* proteins are quite similar (24). Most of the amino acid alterations between the two proteins are conservative (Fig. 3); however, one region (residues 52 to 71) contains major differences that may indicate a region of the protein that is involved in subunit-subunit interactions. Studies with hybrid TraT proteins containing a foreign antigenic determinant also indicate that the regions involved in such interactions are localized, as three of the five hybrids studied, with insertion of the foreign sequence at residues 125, 180, and 200, retained the ability to assemble into oligomers (Taylor et al., in press).

The TraT permeability mutants are the only examples of this class of protein mutants for which the amino acid alterations are known. Therefore, they should be invaluable tools for analysis of the molecular organization of the permeability barrier function of the outer membrane. The mutant TraT proteins that cause the membrane permeability phenotype have amino acid alterations in the second hydrophobic domain (residues 117 to 135), and thus far, only one mutant TraT with similar properties has been isolated with an amino acid alteration in the first hydrophobic domain (Gly-107 to Arg; our unpublished results). However, it is likely that the underlying molecular cause of outer membrane permeability is the same for both domains, as, in each case, charged residues are generally involved in disruption of membrane integrity.

GENETICS OF *traT* EXPRESSION

The *tra* operon of F-like plasmids contains 23 genes, which, with the exception of *traJ* and *traM*, are thought to belong to one transcriptional unit (35, 103). The *traJ* gene is the regulator of this operon and acts positively to promote transcription of the other *tra* genes. The *traT* gene is located after the other surface exclusion gene, *traS*, and precedes *traD* (35). Recent work, however, has uncovered several internal promoters, suggesting that control of expression of the *tra* genes may be more complex than originally thought.

The sequencing studies of Ogata et al. (68) suggested that the *traT* gene of the R100 plasmid has its own promoter, which is independent of *traJ*. This finding suggested that the same might be true of the F *traT* gene. However, experiments to investigate the expression of this gene have given contradictory results.

Mutations in the F *traJ* gene abolished surface exclusion, whereas the amount of the TraT protein decreased only three- to fourfold (72). This suggests that there is a threshold level below which surface exclusion does not take place and, additionally, that a separate *traT*-specific promoter exists. However, the possibility that the *traT* mRNA is unusually stable was not excluded. The decrease in surface exclusion in *traJ* mutants was thought to be due principally to the lack of the *traS* gene product. A strong RNA polymerase-binding site has also been found in the vicinity of the F *traT* gene (52). However, when *galK* fusion studies were made with an F-derived DNA fragment, which should have carried the proposed *traT* promoter, no enhancement of *galK* expression was detected (61). A construction containing the F *traG'*, *traS*, *traT*, and *traD* genes in a lambda transducing phage showed repression of all these genes by the lambda repressor (57), and the authors concluded that there is no *traT*-specific promoter in the F plasmid. The observed production of TraT in *traJ* mutants is due either to the stability of the TraT mRNA or to a small amount of *traJ* gene product present in leaky mutants.

In contrast, later studies (11, 73) confirmed that synthesis of the F TraT protein can occur in the total absence of the *traJ* gene product. Moreover, kinetic studies of the expression of the entire operon showed that large amounts of the TraT protein were produced before any other *tra* gene products, including *traJ*, were detected (11). It is possible, therefore, that the earlier *galK* studies failed to detect a *traT*-specific promoter because of its weakness and also because the cloned fragment may have lacked sequences necessary for the stabilization of *traT* mRNA.

With the availability of the complete nucleotide sequence of the region of F containing *traS* and *traT* (37), promoters for both *traS* and *traT* have become apparent. These promoters, P_S and P_T, respectively, are active in fusions with

galK (37). The start and termination points of *traT* transcription were precisely mapped by S1 nuclease protection studies (31), which showed that P_T was located within the *traS* gene. RNA stability experiments in the same study indicated that the mRNAs for both *traS* and *traT* were unusually stable. Therefore it seems that the *traJ*-independent expression of the F TraT protein is due partially to a *traJ*-independent promoter and partially to a stable mRNA. In view of the promoter probe studies, however, it is likely that the *traT* promoter is weaker than the *traS* promoter, which precedes it (31). Therefore, most of the *traJ*-independent transcription comes from the *traS* promoter upstream.

The *traT* gene of the F*olac* plasmid also seems to have its own promoter, since the corresponding protein can be produced in high yield from the cloned gene without the use of a vector promoter (24). Potential promoters have been identified in the sequences upstream of the sequences coding for the TraT proteins of the *S. typhimurium* and *Y. enterocolitica* virulence plasmids (91; China et al., in press). However, the regulation of expression of these genes is clearly different from that of the F *traT* gene. The pSLT TraT protein is produced at extremely low levels under laboratory conditions (76), and expression of the pYV-encoded TraT protein is both Ca^{2+} dependent and temperature sensitive. Interestingly, expression of the F TraT protein is also temperature dependent (although not, of course, Ca^{2+} regulated), with essentially no protein detectable at 25°C (84). An interesting possible explanation for this effect is that expression of *traT* is altered by the extent of DNA supercoiling (19).

ROLE OF THE TraT PROTEIN OF F-LIKE PLASMIDS IN SERUM RESISTANCE AND PATHOGENESIS

Bacterial resistance to serum complement has often been associated with the presence of F-like plasmids (6, 22, 74, 75, 96, 104). In general, the observed increase in resistance caused by the plasmid is only moderate and the resistance level of rough strains bearing such plasmids, e.g., *E. coli* K-12 carrying an F-like plasmid, is not as high as that of smooth *E. coli* strains (93). Therefore, the increase in resistance is detected only if the serum concentration used is adjusted in accordance with the basic sensitivity level of the bacterial strain studied. Moreover, the resistance is dependent on the serum source, which makes it difficult to compare the results from different research groups. Therefore, Ogata and Levine (67), who compared the effect of the R100 plasmid on resistance of *E. coli* K-12 to different sera, found virtually no effect with human serum but found clearly increased resistance when guinea pig or rabbit serum was used. The plasmid gene responsible for the increased resistance has been identified as *traT* for both R100 and R6-5 (58, 68).

The colicinogenic plasmid ColV2-K94, carried by many clinical isolates of *E. coli*, was first shown to contain a serum resistance gene, designated *iss*, which was located outside the *tra* region of the plasmid (6). Later, however, a *traT* gene was identified on ColV2-K94 by DNA hybridization (15). It was shown that both *traT* and *iss* contribute to the resistance caused by ColV2-K94 in guinea pig serum, even though the effect of *traT* could be demonstrated only when *iss* was deleted or when *traT* was present on a multicopy plasmid (15). Therefore, serum resistance mediated by ColV2-K94 seems to be due mostly to the *iss* gene. The nucleotide sequence of the *iss* gene has recently been determined and, as expected, shows no significant homology to *traT* (14).

The precise mechanism of the serum resistance caused by the *traT* gene product is not known. It seems, however, that it does not involve degradation or nonproductive binding of complement components (94, 95). Assays for consumption of the different complement components revealed no significant differences in the amounts of C6, C7, C8, or C9 bound to *traT*$^+$ or *traT*-lacking *E. coli* K-12 cells in 10% rabbit serum (7). The presence of the *traT* gene did not reduce the binding of C3b to wild-type *E. coli* in human serum (4). Therefore, the role of the *traT* gene product in serum resistance is more likely to be inhibition of the correct assembly or membrane insertion of the membrane attack complex of complement (94). A similar mode of function has been suggested for the protein of *Neisseria gonorrhoeae* (40–42); however, direct evidence is lacking in both cases.

The TraT protein has also been reported to reduce the susceptibility of wild-type *E. coli* cells to phagocytosis by mouse peritoneal macrophages (4). In this study cloned *traT* genes were used, and, in contrast to results obtained in serum resistance experiments, in which a protective effect could be detected when single-copy-number plasmids were used, inhibition of phagocytosis by TraT was dependent on the copy number of the plasmid.

The ability to resist the bactericidal activity of animal sera is common among invasive pathogens (23, 50, 70, 92). Because of this, large studies were undertaken to screen for the presence of the *traT* gene in clinical isolates of *E. coli* and other gram-negative bacteria (8, 59). A TraT-specific monoclonal antibody was used to screen *E. coli* strains, isolated from patients with septicemia, upper urinary tract infections, or diarrhea and from the feces of healthy individuals, for the presence of the TraT protein in their outer membrane (8). TraT was found in a significantly higher proportion of the clinical isolates (51 to 56%) than the fecal strains (38%); the difference was, however, rather small. The authors also reported a 66 to 88% association between the production of TraT and the presence of a capsule of serotype K1 (8).

Montenegro et al. (59) screened clinical isolates by colony hybridization with a probe carrying most of the *traT* gene. They found *traT* in *Salmonella*, *Shigella*, and *Klebsiella* isolates as well as in clinical *E. coli* isolates, but not in *Pseudomonas*, *Aeromonas*, *Plesiomonas*, *Enterobacter*, *Proteus*, *Acinetobacter*, *Citrobacter*, or *Serratia* isolates or the two *Yersinia* strains tested. In all the positive cases the presence of *traT* was associated with the presence of a large plasmid. Surprisingly, however, no correlation between *traT* and the serum resistance of the strains was found. This was also shown by Kanukollu et al. (44), who examined a large number of strains of the family *Enterobacteriaceae* isolated from human extraintestinal infections.

traT AS A VIRULENCE PLASMID GENE

Virulent *S. typhimurium* strains carry a large (100-kilobase) plasmid, which is necessary for virulence (29, 30, 43). This plasmid was first shown by colony hybridization to contain a *traT*-like gene (59). Later, Rhen et al. (76) cloned the gene from the virulence plasmid and demonstrated that it produced an outer membrane protein recognized by TraT-specific antibodies. The *S. typhimurium* virulence plasmid is nonconjugative (M. F. Edwards, Ph.D. thesis, Stanford University, Stanford, Calif., 1985) and does not express any other *tra* functions. Our unpublished hybridization experiments indicate that the virulence plasmid does not carry any of the *tra* genes preceding *traT* and also probably lacks the *tra* genes following *traT*. The clones used as hybridization

probes, pRS27 and pRS29 (3, 83), covered a large part of the *tra* operon.

The *traT* gene of *S. typhimurium* plasmid has been sequenced (91) and found to be closely related to the corresponding genes of F and R100 (see above). Additionally, hybridization studies have shown that the *traT* gene sequence was also found in *Salmonella* serovars *choleraesuis*, *enteritidis*, *johannesburg*, *kottbus*, and *pullorum* (46).

As with the previously described TraT proteins of F-like plasmids (58, 68), the TraT protein produced by the virulence plasmid of *S. typhimurium* increased the resistance of both *E. coli* K-12 and *S. typhimurium* to guinea pig serum (77). However, the *traT* gene does not seem to be needed for the growth of *S. typhimurium* intracellularly in the liver and spleen of the mouse (77), a property known to be dependent on genes of the virulence plasmid (30). In contrast, recent results suggest that the *traT* gene of *S. typhimurium* might affect the survival of the bacteria within guinea pigs during the blood phase of infection (P. Riikonen, S. Sukupolvi, P. H. Mäkelä, and M. Rhen, unpublished data). The relative contribution of the TraT protein to pathogenic potential may depend on the *Salmonella* serovar studied; e.g., *S. enteritidis* strains also harbor a *traT*-like gene (46), but, in contrast to results with *S. typhimurium*, it does not appear to be important for these bacteria, as they do not need the virulence plasmid for resistance to guinea pig serum (33).

Recently, a *traT* gene coding for an outer membrane lipoprotein, designated YlpA, has been found on the virulence plasmid of *Y. enterocolitica* (China et al., in press). The *Yersinia* plasmid, like that of *S. typhimurium*, is not self-transmissible (5) and probably does not contain any other *tra* genes. Interestingly, the gene is flanked by virulence-associated *yop* genes, and production of the protein requires incubation of host bacteria at 37°C in a medium deprived of Ca^{2+} ions; these conditions are known to induce the biosynthesis of the Yop virulence proteins (5; China et al., in press). These findings suggest that the YlpA protein might be involved in pathogenicity, particularly as preliminary results of a previous study showed that a *Y. enterocolitica* strain with Tn*3* inserted in the gene appeared to be less virulent than the wild-type strain (5). However, the same study also showed that the mutant strain had no detectable difference in its ability to survive in human serum, and hence the role of the YlpA protein in virulence remains unclear at the present time.

The fact that *traT* sequences are found in several virulence-associated plasmids raises the question of how a gene, located within the transfer operon of conjugative F-like plasmids, can be found in isolation in these nonconjugative plasmids. One possibility is that the other *tra* genes were originally present in ancestors of the virulence plasmids but were gradually lost. In this case, the finding that two distinct virulence plasmids have both retained the *traT* gene strongly suggests that the presence of this gene confers a selective advantage on host cells. The most obvious function of the gene, at least in *Salmonella* species, would be serum resistance; however, other possible roles should not be discounted. An alternative explanation is that the gene was picked up from a coresident F-like plasmid (e.g., an antibiotic resistance or colicinogenic factor), and, in this respect, it may be significant that at least one *traT* gene (that of R100) contains an IS*2* insertion sequence immediately upstream of the coding region (68).

SURFACE EXCLUSION

Surface exclusion is defined as reduced ability of a strain carrying a conjugative plasmid to act as a recipient in conjugation with cells carrying identical or closely related plasmids. Therefore, cells carrying the F plasmid typically have a 100- to 300-fold reduction in their ability to act as recipients relative to an F^- cell (1, 100).

Two plasmid-borne genes, *traS* and *traT*, are responsible for surface exclusion by F-like plasmids, and although their contributions are synergistic, their gene products operate through quite separate mechanisms (1, 2, 45). The product of the *traS* gene is an inner membrane protein that is thought to act by inhibiting the triggering of conjugal DNA metabolism. In contrast, the TraT protein blocks conjugation at an earlier stage, before the cells have formed stable mating aggregates (1).

Experiments with *traS* or *traT* mutants of the low-copy-number plasmid pRS31 have shown that much of the surface exclusion effect is attributable to the *traS* gene product. As recipients, *traS*$^+$ *traT* cells were only 16 to 26 times less effective than control cells, whereas *traS traT*$^+$ cells were 90 to 215 times less effective (1). However, the degree of surface exclusion due to the TraT protein is gene dosage dependent, and its contribution to surface exclusion can be increased severalfold when the *traT* gene is present on a plasmid with a higher copy number than that of the F plasmid (1).

Four surface exclusion groups, termed Sfx_I through Sfx_{IV}, have so far been identified for the F-like plasmids (101). A plasmid belonging to one surface exclusion group will transfer DNA at essentially normal frequencies to cells carrying a plasmid from any of the three groups. Therefore, an F*his* plasmid (Sfx_I) can be transferred with no significant surface exclusion to a recipient carrying the R100-1 plasmid (Sfx_{IV}), whereas transfer is inhibited more than 300-fold when the F*his* plasmid is crossed with a recipient cell carrying a plasmid of the same surface exclusion group, such as F*lac* (100, 101). This observation strongly suggests that both the TraS and TraT proteins are specific for each group of plasmids.

Recently, the amino acid sequences of TraT proteins from at least three different surface exclusion groups have become available following the cloning and sequencing of their genes, and it has therefore become possible to identify amino acid changes that may be responsible for surface exclusion specificity (24, 37, 68; Taylor et al., unpublished). Interestingly, the mature forms of the proteins determined by using the F, ColB-K98, and R100 plasmids (belonging to surface exclusion groups I, II, and IV, respectively) differ by only single amino acid substitutions that are clustered in the same region of the protein (Fig. 3), strongly suggesting that amino acids 116 to 120 determine the specificity of the TraT protein in surface exclusion. This has been confirmed by domain-swapping experiments: genetic alteration of the region of the R6-5 protein to the corresponding region in the ColB-K98 protein, resulted in switching in surface exclusion specificity from Sfx_{IV} to Sfx_{II} (Taylor et al., unpublished). Although the specificity region is flanked by hydrophobic residues, it is hydrophilic, which raises the possibility that it is surface exposed and hence able to interact with external agents.

At first glance it is difficult to reconcile the subtle difference between the F and R100-1 TraT proteins (in effect, the two proteins differ by a single methyl group) with the differences in surface exclusion that they exhibit in vivo. However, secondary structure predictions suggest that the

presence of Gly rather than Ala at position 119 (in the protein of F) creates an additional β-turn, which may radically alter the structure of the protein in this region (13).

The precise mechanism of action of the TraT protein in surface exclusion is poorly understood. The initial stages of the conjugation process involve interactions between the pilus of the donor cell and the surface of the recipient which are thought to lead to pilus retraction, bringing the two cells into close contact (3, 36, 82). Subsequently, the contacts between the mating cells are stabilized and transfer of DNA proceeds through an ill-defined DNA channel (35, 102). It has been known for some time that cells expressing the TraT protein do not form stable mating aggregates with donor cells and hence do not give rise to the characteristic clumping of cells observable in normal mating cultures (1). This suggests that the TraT protein masks a receptor for the sex pilus on the surface of the recipient cell, thus preventing stable attachment of the pilus to the recipient.

The nature of the pilus receptor is still a matter of some uncertainty. In the F plasmid, a possible candidate is the OmpA protein, which is required on the recipient cell surface for efficient conjugation in liquid media (3). OmpA mutants that are Con^- have a Gly-to-Asp alteration at position 154, an amino acid residue known to be important for binding of the OmpA-specific phage Ox2 (60, 78). Moreover, Riede and Eschbach (79) have now presented evidence suggesting that the TraT protein also interacts with the protein, masking a normally surface-exposed region that is necessary for efficient binding of a different OmpA-specific bacteriophage, K3 (78). These results suggest that the F TraT protein may function in surface exclusion by preventing the interaction of the OmpA protein with a component from the donor cell, presumably the sex pilus.

The pilin proteins of F-like plasmids are highly conserved in sequence (26), which could imply that they interact with the same outer membrane component. However, this assumption now seems less likely, as F-like plasmids such as R100-1 transfer at normal frequencies to *ompA* mutant strains that prevent the transfer of F DNA (53). Additionally, studies on bacteriophage host range mutants have shown that single-amino-acid alterations in the adhesin proteins of the phages allow completely different outer membrane proteins to serve as phage receptors (79). Therefore, it is probable that more than one component of the outer membrane can serve as a pilus receptor and that the particular receptor used depends on the pilus type.

Studies with lipopolysaccharide-deficient mutants suggest that lipopolysaccharide also plays an important role in pilus receptor function, as *E. coli* and *S. typhimurium* strains with heptose-deficient lipopolysaccharide are very poor recipients in F-mediated conjugation experiments (32, 80). Additionally, the presence of complete lipopolysaccharide molecules (i.e., with O [somatic] side chains) in *S. typhimurium* inhibits conjugal transfer of DNA (99), presumably because O side chains sterically hinder the access of the pilus to its receptor. This observation suggests that the receptor is relatively near the surface of the outer membrane and hence might also be masked by the highly exposed TraT protein.

More recently, Minkley and Willetts (57) have proposed that the TraT protein binds to the pilus tip. In this model, the oligomeric form of the protein would interact with the tip of a pilus specified by a plasmid of the same surface exclusion group but not with pili specified by other F-like plasmids. This modified model explains the finding that donor cells expressing mixed pili, as a result of the presence of plasmids with different surface exclusion and pilus genes, are not subject to surface exclusion when either of the same plasmids is present in the recipient cell (100). Moreover, sequence analysis of the pilin proteins from several F-like plasmids shows that there is a very good correlation between differences in the amino-terminal region of the pilins and the surface exclusion specificities of different F-like plasmids (26). However, it is not clear from the model how a donor cell would prevent its pili from binding nonproductively to TraT protein molecules on its own cell surface. F pili are about 2 μm long on average, but may be up to 20 μm long (9, 10). Therefore, they have the potential to loop back and interact with components on the donor cell elaborating them. Possibly, the interactions between the pilus tip and the TraT molecule are relatively weak; this would subsequently allow the pilus to interact with TraT molecules on the cell surface of the recipient.

CONCLUDING REMARKS

Ten years ago essentially nothing was known about the structure of the TraT protein, and its role in surface exclusion had only recently been discovered (1). Since then, significant information on its molecular organization has become available, although much remains to be learnt about its biological functions, particularly its role in virulence. The unexpected finding that the gene occurs, separate from other *tra* genes, in the virulence-associated plasmids of *Salmonella* and *Yersinia* species provides further hints that surface exclusion is not the only biological function of the protein; however, the evidence is still circumstantial and further work is needed to clarify the issue.

Because surface exclusion is essentially a recognition phenomenon, in which plasmid-bearing cells are tagged as a result of the presence of a specific TraT protein on their cell surface, it is not too fanciful to view the protein as a primitive type of major histocompatibility protein (25), allowing such cells to discriminate self from nonself. The available evidence also suggests that the TraT lipoprotein is a major surface-exposed protein in *E. coli* K-12 and, further, that its structure is probably based on design principles that differ markedly from those of other outer membrane proteins. It is hoped that these unusual features will prompt further studies to elucidate the structure and function of the protein at the molecular level.

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